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Genetic and Physical Interactions Involving the Yeast Nuclear Cap-Binding Complex

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Yeast strains lacking the yeast nuclear cap-binding complex (yCBC) are viable, although impaired in growth. We have taken advantage of this observation to carry out a genetic screen for components that show synthetic lethality (SL) with a *cbp20*-Δ *cbp80*-Δ double mutation. One set of SL interactions was due to mutations that were complemented by components of U1 small nuclear RNP (snRNP) and the yeast splicing commitment complex. These interactions confirm the role of yCBC in commitment complex formation. Physical interaction of yCBC with the commitment complex components Mud10p and Mud2p, which may directly mediate yCBC function, was demonstrated. Unexpectedly, we identified multiple SL mutations that were complemented by Cbf5p and Nop58p. These are components of the two major classes of yeast small nucleolar RNPs, which function in the maturation of rRNA precursors. Mutants lacking yCBC were found to be defective in rRNA processing. Analysis of the yCBC deletion phenotype suggests that this is likely to be due to a defect in the splicing of a subset of ribosomal protein mRNA precursors.

Most eukaryotic organisms have a complement of three specialized RNA polymerases (pol I, pol II, and pol III) responsible mainly for rRNA, mRNA, and tRNA synthesis, respectively. pol II transcripts have characteristic 5′ ends consisting of a 7-methylguanosine cap structure attached by a 5′-5′ phosphotriester linkage to the first encoded nucleotide of the transcript (66). Aside from providing protection against 5′-to-3′ exonuclease activities, the cap structure plays important roles at multiple steps in the function of pol II transcripts. In vertebrates, the cap has been shown to stimulate pre-mRNA splicing (30, 37, 38, 57), pre-mRNA 3′ end formation by cleavage and polyadenylation (11, 15, 19, 23), export from the nucleus of U small nuclear RNAs (snRNAs) (22, 32), and the initiation of translation (67).

The effect of the cap in mRNA translation is mediated by eukaryotic initiation factor 4F (eIF-4F), a multicomponent complex whose cap-binding subunit is eIF-4E (67). In contrast, the nuclear functions of the cap are all thought to be mediated by CBC, the nuclear cap-binding complex. CBC consists of a heterodimer of two proteins, CBP80 and CBP20 (30, 31, 33, 34), and evidence from in vivo and in vitro experiments supports its role in both pre-mRNA splicing and U snRNA export (30, 31, 44). CBC associates with the cap structures of pre-mRNA and nuclear mRNA in vivo and accompanies mRNA through nuclear pore complexes to the cytoplasm (78). There is, however, no evidence that CBC plays an important role in the nuclear export of mRNA, in contrast to its function in the export of U snRNAs (31, 32).

While the in vitro evidence for the function of CBC in cleavage and polyadenylation is direct (15), the current in vivo data on this topic are less definitive. It has, however, been observed that in cells whose largest pol II subunit is truncated, transcripts are not efficiently capped (9, 48), and the majority of the RNAs produced thus lack a high-affinity CBC binding site. Such uncapped transcripts are neither spliced nor cleaved and polyadenylated efficiently (49). Although the latter effect may reflect direct interaction between the carboxy-terminal domain of the pol II subunit and the cleavage and polyadenylation machinery (24, 49), the lack of CBC binding to the transcripts may also contribute to the inefficiency of their 3′ end formation.

CBC has been identified in yeast (10, 20, 43). Yeast CBP80 (yCBP80) is encoded by the *GCR3* gene (75), and yCBP20 is encoded by *MUD13* (10, 20). In contrast to the data for multicellular eukaryotes discussed above, the data reported on CBC function in yeast relate only to pre-mRNA splicing. *mud13* and *gcr3* strains exhibit reduced splicing of a reporter gene that carries a nonconsensus 5′ splice site (10) or a nonconsensus sequence in the branchpoint region (15a). In vitro splicing is also decreased in extracts that were biochemically depleted of CBC (43) or extracts from a *mud13* strain (10). Yeast strains that lack RNA capping activity do not show obvious defects in mRNA cleavage or polyadenylation (16, 63), and extracts from yeast cells that lack CBC do not exhibit defects in 3′ end formation in vitro (15a). It is unclear whether yeast U snRNAs resemble their vertebrate counterparts in being transported out of the nucleus during maturation, and yCBC function in U snRNA export has therefore not yet been tested.

In pre-mRNA splicing, yCBC and human CBC (hCBC) play analogous roles. They increase the efficiency with which U1 snRNP binds to the cap-proximal 5′ splice site and thus increase the rate of recognition and splicing of the cap-proximal intron (10, 43, 44). In biochemical terms, this manifests itself as an increase in commitment complex formation (64) in the presence of CBC (10, 43). Although the functions in pre-mRNA splicing of yCBC and hCBC are therefore likely to be mechanistically related, the detailed mechanism by which CBC exerts its role is not known. Attempts to demonstrate direct interaction between hCBC and human U1 snRNP were unsuccessful (44), resulting in the hypothesis that one or more unknown factors mediate the CBC-dependent increase in U1 snRNP-5′ splice site interaction.

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To identify this intermediary and, more generally, to obtain additional insight into CBC function, a genetic analysis of CBC in *Saccharomyces cerevisiae* has been undertaken. Twelve distinct genes whose mutation leads to lethality in the absence of yCBC are identified. Complementation of these defects reveals that the great majority of these genetic interactions can be explained on the basis of yCBC function in the commitment complex assembly step of pre-mRNA splicing. Further, evidence of physical interaction between yCBC and two commitment complex components, Mud2p and Mud10p, is presented.

MATERIALS AND METHODS

DNA constructs. To construct pHT80, the *GCR3* gene was PCR amplified from genomic DNA from position −800 before the ATG to position 290 after the stop codon of yCBP80. The amplified DNA was digested with *Sma*I and *Sal*I and cloned into the same sites of the polylinker of pHT4467, a single-copy plasmid with *ADE2* and *URA* markers. The functionality of the gene was checked by its ability to restore growth and splicing efficiency to wild-type levels in the *cbp80*-Δ strain.

pSEY8-yCBP20, a full-length yCBP20 clone from a yeast genomic library (20), was digested with *Hin*dIII and repaired with Klenow enzyme to obtain a 1-kbp fragment that was cloned using the *Sma*I site of pHT4467 or pHT80 to generate pHT20 and pHT8020, respectively. The expression of yCBP20 and yCBP80 in these pHT4467-derived plasmids was assayed by growth restoration of the *cbp* disrupted strains and by Western blotting using extracts isolated from *cbp20*-Δ, *cbp80*-Δ, and *cbp20*/*80*-Δ strains transformed with pHT20, pHT80, and pHT8020 respectively.

To construct YEp20, the open reading frame (ORF) of *MUD13* was amplified from pSEY8-yCBP20 (20). The fragment was digested with *Bam*HI and *Hin*dIII and cloned in the same sites under the *GAL10* promoter of YEp51, a multicopy plasmid with a *LEU* marker (62). Similarly, to construct YEp80, the ORF of yCBP80 was amplified with oligonucleotides that avoid intronic sequences. The PCR fragment was end repaired and cloned in the *Sma*I site of pBluescript SK+ (Stratagene) to generate pBS-*GCR3*. This plasmid was then digested with *Eag*I and *Bgl*I; the ends were repaired with Klenow enzyme and digested with *Sal*I. The fragment containing *GCR3* was cloned under the control of the *GAL10* promoter in YEp51. The expression of yCBP80 and yCBP20 in YEp51-derived plasmids was analyzed by growth rate restoration and by Western blotting.

Deletion of yCBC genes. The techniques used for growing yeast are described elsewhere (68). Yeast cells were transformed with DNA by the lithium acetate method (29). Strains used for the synthetic lethal (SL) screen were derived from YJV159 (*MAT***a** *ade2 ade3 his3 leu2-3,112 trp1 ura3*) (76a).

The *cbp20*-Δ strain (*MAT***a** *ade2 ade3 his3 leu2-3,112 trp1 ura3 ycbp20*/*mud13*::*HIS3*) was obtained by transfection of YJV159 with a linear DNA in which the *HIS3* gene from plasmid Ydp-H (5) had been inserted between the *Bcl*I and *Sna*BI sites of *MUD13*. Transformed cells were grown on SD-His medium, and the *MUD13* deletion was confirmed by PCR amplification of genomic DNA and Southern blotting.

To obtain the *cbp80*-Δ strain (*MAT***a** *ade2 ade3 his3 leu2-3,112 rp1 ura3 ycbp80*/*gcr3*::*TRP1*) and the double-knockout strain (*MAT***a** *ade2 ade3 his3 leu2-3,112 trp1 ura3 ycbp80*/*gcr3*::*TRP1 ycbp20*/*mud13*::*HIS3*), YJV159 and *cbp20*-Δ cells were transfected with a linear DNA carrying a disrupted copy of the *GCR3* gene. The sequence from 20 nucleotides before the ATG to position 2700, just after the last ATG in frame, were replaced by the *TRP1* gene from plasmid Ydp-W (5). Transformed cells where grown on SD-Trp medium, and the *GCR3* deletion was confirmed by PCR amplification of genomic DNA and Southern blotting. The doubling times of these strains were measured, and the expression of yCBP80 and yCBP20 was analyzed by Western blotting. A disruption of *GCR3* was also generated in a *MAT*α strain. A *GCR3* gene fragment from the *Sna*BI site, 200 nucleotides upstream of the ATG, to the *Bgl*II site was replaced by the *HIS3* gene in strain D209 (*MAT*α *ade2 leu2 ura3 his3 rp1*). PCR amplification of genomic DNA and Southern blotting were used to verify the genotype. This strain was crossed with YJV159, the diploid was sporulated and tetrads were dissected. Strain cbp80-Δ α (*MAT*α *ade2 ade3 leu2 trp1 ura3 ycbp80*/*gcr3*::*HIS3*) was identified among the haploid progeny by screening for the desired phenotypes. The doubling time of this strain was 230 min, similar to that of a *cbp80*-Δ strain (Fig. 1).

The *ssd1*-Δ allele was constructed by one-step PCR (4) using the *HIS3* selective marker with integration targeting sequences that precisely delete the entire ORF.

Doubling time and viability tests. Yeast control strain or strains disrupted for *GCR3*, *MUD13*, or both were grown in liquid medium to mid-log phase (optical density at 600 nm of approximately 0.8). Cells were then diluted 20 and 40 times, and optical density was monitored. Doubling time was calculated during logarithmic growth. Dilutions of these strains were also plated on YPD and allowed to grow for 48 h at 30°C (Fig. 1). Similarly, temperature-sensitive LUC mutants (see below for description) were checked for viability. Cells grown to mid-log phase were diluted and shifted to 37°C, and growth was monitored.

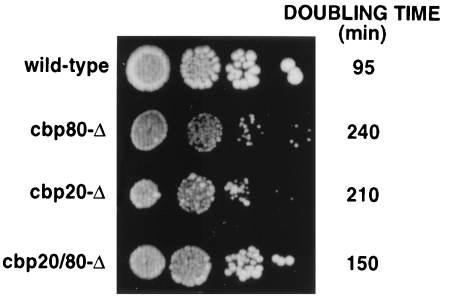


FIG. 1. yCBC affects vegetative growth rate. Strain YJV159 (wild type) was disrupted for *GCR3* (cbp80-Δ), for *MUD13* (cbp20-Δ), or for both (cbp20/80-Δ). These strains were grown to mid-log phase, and four dilutions of each were plated to compare the growth rates. The doubling time of each strain was also assayed in liquid culture and is indicated on the right.

Mutagenesis and the SL screen. An SL screen was performed as described elsewhere (39). The *cbp20*/*80*-Δ strain was transformed with plasmid pHT8020, which encodes yCBP80, yCBP20, Ura3p, and Ade3p. The resulting cells are red due to the *ADE3-ade2* combination. When cells were grown on 4% glucose–YPD medium, a red to white sectoring phenotype was observed, indicating that the strain could lose plasmid pHT8020. Cells plated on 4% glucose–YPD were exposed to 254-nm UV light (Desaga) for 30 s to allow 10% survival; 2.8×10^5 colonies were then screened, and 560 colonies that did not sector were isolated. Of these, 155 did not grow on 5-fluoro-orotic acid (FOA) plates, indicating that they needed pHT8020 to survive; 42 clones showed a red color and FOA sensitivity at different temperatures. To eliminate false positives, these strains were transformed with plasmid YEp80, YEp20, or both. Thirty-two strains were then able to survive on FOA plates, indicating that the phenotype was indeed yCBC related. Most of the strains needed both yCBP80 and yCBP20 to survive on FOA plates, but some showed weak growth with only yCBP20 or yCBP80.

A genetic characterization was then carried out. The mutants were crossed to the *cbp80*-Δ α strain, and in all cases red to white sectoring was observed, indicating that all the mutations were recessive. Diploids were sporulated, and 10 to 12 tetrads were dissected and analyzed phenotypically. In all cases where four spores were recovered, the sectoring phenotype and the FOA lethality segregated 2:2, indicating that the synthetic lethality was probably caused by mutation at a single locus. As some of the mutants showed temperature sensitivity, these crosses also allowed determination of whether temperature sensitivity was linked to synthetic lethality. Finally, the mutant strains were crossed pairwise and plated on FOA plates. Combinations that could not grow were assigned to a complementation group. The 32 strains were sorted into 21 complementation groups named LUC, as they are lethal unless CBC is produced.

Cloning of genes that complement LUC1 to LUC14. Mutants were transformed with a low-copy-number plasmid library (6), using conditions that predict that five times the whole yeast genome was being transformed and a probability of recovery higher than 95%. Transformants were selected on minimal plates. The mutant strains that showed temperature sensitivity linked to the synthetic lethality were grown at 37°C after transformation. The other strains were grown at 30°C, and the sectoring phenotype was allowed to develop after replica plating to YPD–4% glucose, using nitrocellulose membranes (Protran BA 85/20; 0.45-mm pore size; Schleicher & Schuell). Plates were screened, and sectoring colonies were plated on FOA plates. Plasmids containing complementing genomic DNA fragments were recovered from the positives and amplified in *Escherichia coli* XL1-Blue. Retransformation into the mutant strains and rechecking of the sectoring/FOA or temperature resistance phenotype was performed. Insert DNA boundaries were sequenced and compared to the MIPS (Munich Information Centre for Protein Sequences) yeast database (50–52) to define the complementing region. As the average insert size of the library was 10 kb, several genes were usually present in the inserts. When several positives were isolated from a single mutant strain, the overlapping region of the inserts helped to define the complementing ORF. When two or more genes were still partially or totally included in the overlap, they were cloned independently in pRS315, a single-copy plasmid with a *LEU* marker (69) and retransformed in the mutant strain. Plasmids expressing Mud1p, and Mud2p, and SmD3p were kindly provided by M. Rosbash and B. Séraphin. Sectoring phenotype and FOA (or temperature) resistance were used to define the complementing ORF.

yCBC column preparation and binding assays. The yCBC column was prepared as described previously (20, 43). After preparation, 10 μl of the column was boiled in sodium dodecyl sulfate (SDS) sample buffer without reducing agents, and the proteins bound to it were separated by electrophoresis in an SDS–12% polyacrylamide gel and stained with Coomassie blue dye. Only two proteins were detected. Western blotting analysis identified them as yCBP80 and yCBP20. An unrelated antibody column was incubated with yeast extracts under conditions similar to those used with a negative control.

The U1 snRNP proteins isolated in the screen and Mud2p were labeled with

[^{35}S]methionine in an in vitro T7 coupled transcription-translation reaction (TNT-T7 kit; Promega). The T7 promoter-containing template was obtained as a PCR amplification product. The 5′ oligonucleotides used contained the T7 promoter sequence followed by 20 nucleotides around the ATG region of the ORF. In the case of *MUD1*, the 5′ oligonucleotide was longer and included the sequence from the ATG to the sixth nucleotide after the intron. The 3′ end oligonucleotides used contained the sequence complementary to the last 20 nucleotides of the ORF. The PCR was done with standard conditions for cloned *Pfu* polymerase (Stratagene), 1 μg of plasmid DNA, and 20 to 25 cycles. The PCR product was purified by using a Qiaquick PCR purification kit (Qiagen); 0.5 μg of the amplified product was incubated in a 50-μl reaction mixture with the reticulocyte lysate TNT-T7 mix (Promega) that couples transcription and translation. The labeled proteins were then diluted to 500 μl with phosphate-buffered saline-8.5% glycerol and centrifuged through a Nanosep 30K filter (Pall Filtron) at 10,000 rpm (Biofuge A; Heraeus) at 4°C until a 10-fold concentration was achieved. This step was repeated twice to eliminate the unincorporated [^{35}S]methionine.

The control and yCBC columns were washed in binding buffer (50 mM Tris [pH 7.5], 150 mM NaCl, and 10% glycerol in complete protease inhibitor cocktail from Boehringer Mannheim); 15 μl of control or yCBC column beads (corresponding to 1.5 μg of yCBC) was mixed with 3 μl of labeled proteins in a final volume of 200 μl of binding buffer. The mixture was rotated for 2 h at 4°C. The beads were pelleted, and the supernatant was recovered. The beads were washed three times with 1 ml of binding buffer. Supernatant and pellet fractions were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and visualized by fluorography.

Northern hybridization. RNA was extracted as described previously (71), separated by electrophoresis, and transferred to a filter. For hybridization to snoRNAs, the oligonucleotides anti-U3 (5′-CUAUAGAAAUGAUCCU), anti-U14 (5′-TCACTCAGACATCCTAGG), anti-snR10 (5′-CUIUUAAAUUUIC IUU), snR3 (TCGATCTTCGTACTGTCT), and anti-snR30 (ATGTCTGCAG TATGGTTTTAC) were used. Oligonucleotide anti-U3 is largely composed of 2′-*O*-methyl RNA.

For hybridization to mRNAs, the oligonucleotides used were anti-ACT1 (5′-TCTTGGTCTACCGACGATAGATGGGAAGACAGCA), anti-RPS9A (5′-G TTGTATACTTTTGTATTTCT), anti-RPS9B (5′-TGTTGCTTAGTCTTAGT TG), anti-RPS11A (5′-CTTGCTGGTTGCTTAATTT), anti-RPS11B (5′-TCC CTGGCTTGATACGTT), anti-RPS3 (5′-GACACCGTCAGCGACTAG), anti-RPS10A (5′-GCTTGGTTGAAATCCTTC), anti-RPL16A (5′-CTCGATTTGT TCTTCACCTTC), anti-RPL16B (5′-CCAACCAACCAACAATAATAC), anti-RPL22A (5′-CTTAATCTGTTGTTTTGGTGG), anti-RPL22B (5′-GTGGTTG ATATTTGTGAAACG), anti-RPL10 (5′-CTGTAACATCTAGCTGGTC), anti-RPL30 (5′-GGTTGATAGAATCTTGGGAT), anti-RPL28 (5′-GTGCTTTCT GTGCTTACCGATACGACCTTTACCG), and anti-RPL25 (TTTCTTAGCG GCAGTAGCC).

For pre-rRNA hybridization, oligonucleotides depicted in Fig. 4A were used: 001 (5′-CCAGTTACGAAAATTCTTG), 002 (5′-GCTCTTTGCTCTTGCC), 003 (5′-TGTTACCTCTGGGCCC), 007 (5′-CTCCGCTTATTGATATGC), 008 (5′-CATGGCTTAATCTTTGAGAC), and 013 (5′-GGCCAGCAATTTCAAG TTA).

RESULTS

It was previously determined that *GCR3*, which encodes yCBP80, and *MUD13*, which encodes yCBP20, are not essential genes in *S. cerevisiae* (10, 75), strongly suggesting that yCBC is not necessary for yeast vegetative growth. To further investigate this, the growth of yeast strains lacking *GCR3* (*cbp80*-Δ), *MUD13* (*cbp20*-Δ) or both genes (*cbp20*/*80*-Δ) was analyzed. As shown in Fig. 1, strains that lack either CBP80 or CBP20 individually grow slowly at 30°C on rich medium either on plates or in liquid culture. The growth defects are similar at higher (37°C) or lower (23°C) temperatures (data not shown). Analysis of extracts made from the strains by Western blotting showed that while the *cbp20*-Δ strain accumulated amounts of yCBP80 similar to those accumulated by the wild-type strain, the *cbp80*-Δ strain accumulated fourfold less yCBP20 than the wild type, suggesting that yCBP20 is unstable in the absence of yCBP80 (data not shown). Since CBP80 and CBP20 need to heterodimerize to bind to capped RNA (30, 31) it was not surprising that the two single-deletion strains showed similar growth defects. It was unexpected, however, that a strain lacking both yCBP80 and yCBP20 (*cbp20*/*80*-Δ) grew better than strains with either single deletion (Fig. 1). This suggested that the production of either CBP80 or CBP20 alone had a dominant negative effect on growth.

TABLE 1. Summary of SL mutations[a]

Mutant	*n*[b]	Gene(s) characterized	Characteristic(s)[c]
LUC1	2	*MUD1*	U1 snRNP
LUC2	2	*MUD2*	U2AF65 homologue
LUC3	1	*NAM8* (*MRE2*/*MUD15*)	U1 snRNP
LUC4	3	*SNU56* (*MUD10*)	U1 snRNP
LUC5	1	*SNU71*	U1 snRNP
LUC6	2	*SMD3*	U snRNP core protein
LUC7	1	*YDL087c*	Metazoan homologues with SR domains
LUC8	2	*CBF5*	snoRNP (H+ACA)
LUC9	5	*NOP58*	snoRNP (C+D)
LUC10	1	*SSD1*	Predicted exonuclease; Ts mutation
LUC11	1	*GCR1*	Transcription factor; Ts mutation
LUC12	1	*SRV2*	Binds actin and adenylate cyclase
LUC13	1	*RPO31*	Subunit of pol III
		YTA1	26S proteasome component
LUC14	1	*YNL206c*	Similar to HMG/SSRP proteins
		SPS18	Unknown function
		SPS19	Peroxisomal 2,4-dienoyl-CoA reductase

[a] Twenty-one complementation groups were isolated in an SL screen with yCBC and named LUC mutants. The strains were transformed with a library of yeast genomic DNA inserts in a low-copy-number plasmid, and plasmids able to suppress the SL phenotype were isolated for 14 complementation groups (LUC1 to LUC14). In 12 of these cases, plasmid analysis allowed the definition of a single open reading frame whose expression reverts the SL phenotype (LUC1 to LUC12). The genes are indicated in each case, and a brief summary of their characteristics is shown on the right. Single genes have not yet been defined for LUC13 and LUC14, but they are contained between nucleotides 5357982 and 547020 of chromosome XV (LUC13) and nucleotides 256819 and 260289 of chromosome XIV (LUC14).

[b] Number of isolates from the same complementation group.

[c] Ts, temperature sensitive; CoA, coenzyme A.

SL interactions. Since a yeast strain lacking both yCBP80 and yCBP20 could grow reasonably well, the double-deletion background served as the basis for a search for genes whose mutation would prove lethal in the absence of CBC (see Materials and Methods for details). In this way, 21 complementation groups that were lethal unless CBC was produced (LUC) were characterized (see Materials and Methods). Fourteen of these complementation groups were rescued by transformation with low-copy-number plasmids containing yeast genomic DNA inserts and named LUC1 to LUC14. In 12 of the 14 cases, the gene responsible for complementation was identified by further analysis, usually through transformation with subfragments of the original DNA insert of the complementing plasmid (Table 1). Many of these complementation groups were represented only once in the collection of SL strains, showing that the screen is unlikely to be saturated.

The LUC genes can be divided into four main groups: (i) those that encode splicing factors that are components of yeast commitment complexes (LUC1 to LUC6); (ii) components of yeast small nucleolar RNPs (snoRNPs) (LUC8 and LUC9); (iii) genes with a function in RNA metabolism that seems unconnected to known CBC functions (LUC10 and LUC13); and (iv) genes with no obvious direct connection to RNA metabolism (LUC11, LUC12, and LUC14).

Genetic interactions between yCBC and splicing factors. *MUD13*, which encodes yCBP20, was characterized on the basis of a mutant allele that caused synthetic lethality when

present in combination with an otherwise viable mutant form of U1 snRNA (10). This finding, together with biochemical data (10, 43), showed that yCBC functioned in the commitment complex assembly step of yeast pre-mRNA splicing. Commitment complexes form on intron-containing pre-mRNAs in the absence of ATP hydrolysis. There are two commitment complexes, CC1 and CC2, both of which depend on U1 snRNP-5′ splice site interaction (64, 65). In addition, CC2 requires interaction between Mud2p and branch point binding protein (BBP), which bind at and near the branchpoint region of the intron, and U1 snRNP bound at the 5′ splice site (1, 2, 7, 65). The identities of genes complementing LUC1 to LUC6 are consistent with the function of yCBC in commitment complex assembly.

LUC1, LUC2, and LUC6 were initially assigned to this category. LUC1/*MUD1* encodes the yeast U1A homologue and also causes synthetic lethality with the truncated U1 snRNA used to identify *MUD13* (46). U1A is a conserved component of U1 snRNP. LUC2/*MUD2* was also found in the truncated U1 snRNA SL screen and encodes the yeast homologue of U2AF65 (1). Both U2AF65 and Mud2p are involved in very early steps of intron recognition (1, 2, 7, 61, 83). LUC6 is complemented by *SMD3*, which encodes one of the core components of the spliceosomal snRNPs (60; see also reference 47). Although not specific for U1 snRNP, in fact an *SMD3* allele that causes synthetic lethality together with a mutant U2 snRNA has previously been isolated (81); mutation of SmD3p could be expected to affect U1 snRNP function at early stages of splicing.

The product of *NAM8*, which complements LUC3, was originally proposed to have a role in mitochondrial RNA splicing (13) and later implicated in meiosis-specific nuclear pre-mRNA splicing events (54). Recently, however, it was identified, along with the products of *SNU56*/*MUD10*, which complements LUC4, and *SNU71*, which complements LUC5, as a novel component of the yeast U1 snRNP (21). These three proteins are all stably associated with yeast U1 snRNA but are not present in vertebrate U1 snRNP (14, 21). Since yCBC and U1 snRNP are both commitment complex components, these findings provide a reasonable explanation for the synthetic lethality that results when these three genes are mutated on a yCBC null background.

YDL087c, which complements LUC7, is not functionally characterized. We have found putative vertebrate homologues by examination of the DNA databases. These homologues have SR domains, characteristic of a large family of metazoan splicing factors (59, 82), consistent with the possibility that the LUC7 SL phenotype is also due to mutation of a protein involved in pre-mRNA splicing. Further characterization of this complementation group is in progress.

Physical interaction between CBC and yeast splicing factors. As described in the introduction, hCBC was shown to stimulate U1 snRNP binding to the cap-proximal 5′ splice site but not to interact directly with U1 snRNP (44). This suggested that one or more mediators of hCBC-U1 snRNP interaction must exist. The analogous role in splicing of yCBC, and the presence of several additional proteins in yeast U1 snRNP compared to its human counterpart (14, 21), suggested that one or more of these proteins might form a direct interaction with yCBC.

To examine this possibility, yCBC was purified from yeast extracts by immune-affinity chromatography using an antibody directed against the N terminus of yCBP80 (20, 43). The column was washed extensively with buffer containing 1 M NaCl; upon subsequent SDS elution, only yCBP80 and yCBP20 were detected by Coomassie blue staining. Mud2p, Snu71p, and Mud10p were synthesized and labeled with [^{35}S]methionine by in vitro transcription and translation and passed over the column. Mud2p and Mud10p were clearly retained on the column (Fig. 2, lanes 1 to 5 and 11 to 15), while Snu71p (lanes 6 to 10), Nam8p and Luc7p (data not shown) were not retained. Since yCBC could not be prepared in recombinant form but had to be purified from yeast extract, and since the Mud2p and Mud10p proteins were produced in reticulocyte lysate, we cannot be certain that the interactions observed are direct rather than mediated by a factor in the lysate, nor is it certain whether posttranslational modifications are required for the interactions. Indeed, Mud10p produced in *E. coli* lysate did not bind to the CBC column (data not shown), suggesting a possible role for modification of this protein in CBC interaction. Despite these caveats, the interactions observed make Mud2p and Mud10p strong candidates for mediating the interactions that allow yCBC to stimulate commitment complex formation. Additional support for this possibility comes from the observation that yCBC and Mud10p were found to interact by the two-hybrid method in yeast (16a).

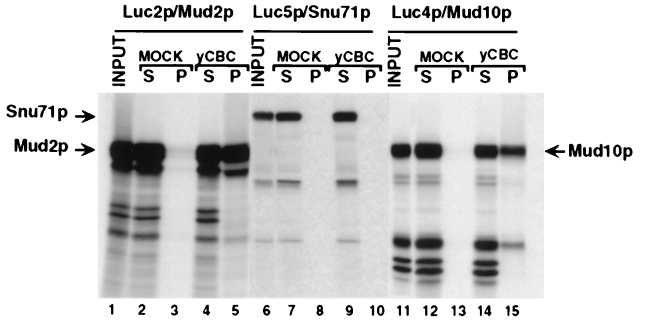


FIG. 2. yCBC interacts with Mud2p and with Mud10p. [^{35}S]methionine-labeled Mud2p/Luc2p (lanes 1 to 5), Snu71p/Luc5p (lanes 6 to 10), and Mud10p/Luc4p (lanes 11 to 15) were incubated with a control column (MOCK) or with a yCBC column as indicated. Samples were fractionated into nonbound supernatant (S) and bound pellet (P) fractions and analyzed by SDS-polyacrylamide gel electrophoresis. In lanes 1, 6, and 11, 25% of the input protein was loaded.

yCBC deletion strains exhibit defects in rRNA processing. LUC8 (two strains) and LUC9 (five strains) were complemented by the *CBF5* and *NOP58* genes, respectively. Cbf5p and Nop58p are both components of snoRNP complexes. The large number of snoRNA species present in eukaryotic cells can be divided into two families on the basis of conserved sequence elements (reviewed in reference 41). Nop58p associates with the box C+D family of snoRNAs (18, 80), most of which function as guides to direct ribose methylation on pre-rRNA (35, 72). Cbf5p is likely to be the rRNA pseudouridine synthase which is guided by the box H+ACA family of snoRNAs to sites of pseudouridine formation on pre-rRNA (17, 40, 55, 72). In addition to their roles in pre-rRNA modification, both classes of snoRNA include members that are critical for pre-rRNA processing at three early cleavage sites designated A_0, A_1, and A_2 (see Fig. 4).

In the presence of functional CBC, the two LUC8 strains and five LUC9 strains were temperature sensitive for growth at 37°C and the LUC9 strains were additionally strongly cold sensitive for growth at 16°C (Fig. 3). Following transfer from 25 to 37°C, the *luc8-sl1* strain showed an inhibition of pre-rRNA processing (Fig. 4 and 5A), while the *luc8-sl2* strain showed a largely nonconditional processing inhibition (Fig. 5A and C). The processing defects resemble those seen in strain depleted of Cbf5p; the 35S pre-rRNA accumulated, while the 32S, 27SA$_2$, and 20S pre-rRNAs were depleted (Fig. 4). Ab-

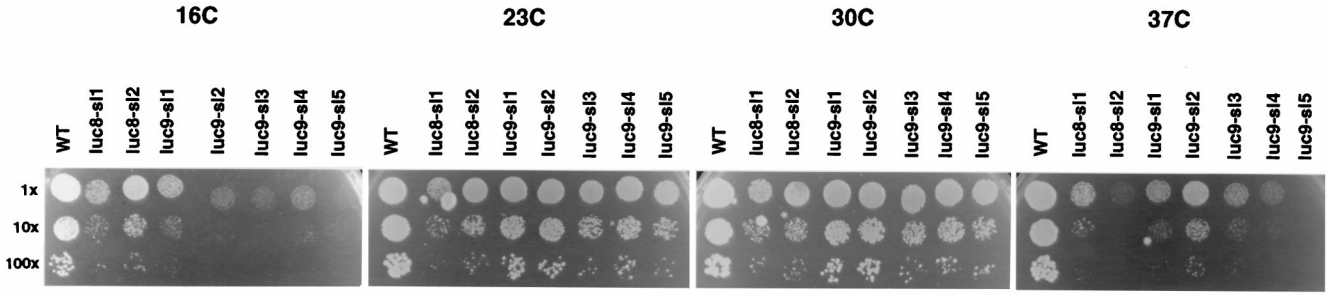


FIG. 3. Growth of the LUC8 and LUC9 strains carrying functional CBC. Dilutions (1- to 10^2-fold) of *luc8* and *luc9* strains along with the wild-type isogenic (WT) control strain were spotted on minimal plates at 16, 23, 30, and 37°C and incubated for 3 days.

errant processing intermediates (the 21S, 22S, and 23S RNAs) were also detected (Fig. 5A and data not shown). These phenotypes are indicative of the inhibition of processing at sites A_0, A_1, and A_2. The LUC9 strains showed a mild pre-rRNA processing defect at 37°C (Fig. 5A) and stronger inhibition of processing following transfer from 30 to 16°C (Fig. 5C). Again, the phenotype was indicative of the inhibition of processing at sites A_0, A_1, and A_2. Similar inhibition is seen in strains genetically depleted of Nop58p (80).

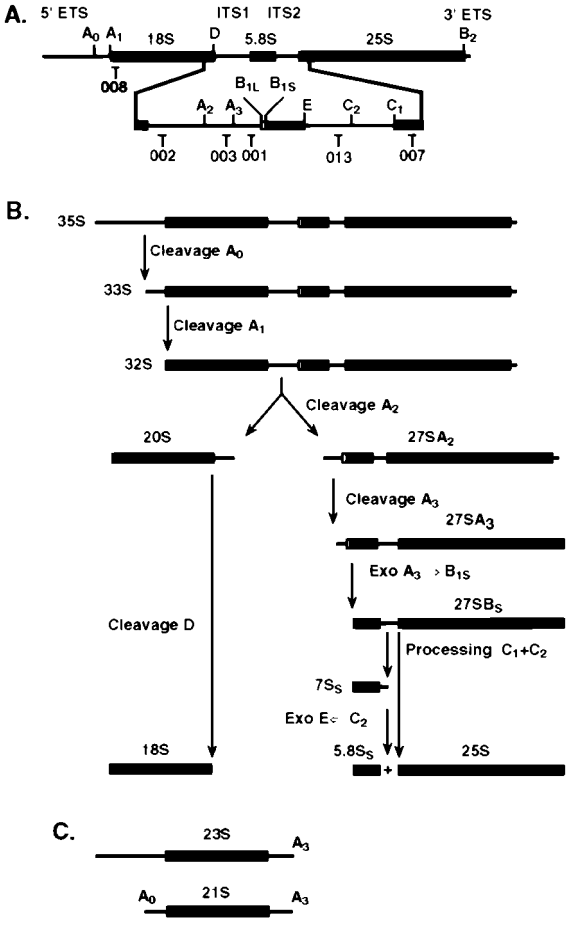


FIG. 4. The yeast pre-rRNA processing pathway. (A) Structure of the pre-rRNA with positions of oligonucleotides used for hybridization. In the 35S pre-rRNA, the mature 18S, 5.8S, and 25S rRNA sequences are flanked by the 5′ and 3′ external transcribed spacers (5′ ETS and 3′ ETS) and separated by internal transcribed spacers 1 and 2 (ITS1 and ITS2). (B) Major pre-rRNA processing pathway in yeast. Note that a minor alternative pathway in ITS1 generates an alternative form of 5.8S rRNA ($5.8S_L$) that is extended 5′ to site B_{1L} (not shown). (C) Structures of the aberrant 23S and 21S RNAs.

The SL strains LUC8 and LUC9, expressing yCBP80 and yCBP20, have both rRNA processing and snoRNA stability defects that are consistent with mutations in *CBF5* and *NOP58*, respectively (Fig. 5). Nop58p is required for the stability of the box C+D class of snoRNAs, while Cbf5p is required for stability of box H+ACA snoRNAs (18, 41a). The *luc8-sl1* strain was found to result in conditional depletion of the box H+ACA snoRNA snR3 at 37°C, while *luc8-sl2* resulted in nonconditional depletion of snR3 (Fig. 5B and D). Depletion of the essential box H+ACA snoRNA, snR30, was substantially less marked at 23 or 30°C (data not shown). None of the LUC9 strains resulted in clear depletion of the box C+D snoRNA, U14 (Fig. 5B and D).

The genetic interaction of CBC with components of both major classes of snoRNP suggested that deletion of CBC might affect pre-rRNA processing. This possibility was tested by Northern hybridization using probes specific for either mature rRNAs (Fig. 6A) or pre-rRNAs (Fig. 6B to F) in strains lacking yCBP80 and/or yCBP20.

Several pre-rRNA species accumulated to abnormally high levels in all three deletion strains; the 35S primary transcript, the 32S pre-rRNA, and an aberrant 21S rRNA (see also Fig. 4). In contrast, the level of the $27SA_2$ pre-rRNA was strongly reduced. The 21S intermediate extends from site A_0 to A_3, and results from cleavage of the 32S pre-rRNA at site A_3 in the absence of cleavage at site A_2. We conclude that A_2 cleavage is particularly inhibited in the mutants. The overall pattern of defects, however, suggests that not only A_2, but also the A_0 and A_1 cleavage events are slowed. Levels of 27SB and 7S pre-rRNAs were not altered (Fig. 6E and F), indicating that the pathway of 5.8S/25S rRNA synthesis is not affected by yCBC deletion (Fig. 4). We conclude that the absence of Cbp20p or Cbp80p inhibits processing at sites A_0, A_1, and A_2, with the greatest effects on A_2. Processing at later steps on the pathway of 5.8S/25S synthesis does not appear to be affected. No clear reduction in the levels of mature 25S or 18S rRNAs was observed (Fig. 6A and G), so the inhibition of mature rRNA synthesis is unlikely to be directly responsible for the slow growth of the *cbp* deletion strains.

Four snoRNA species are required for pre-rRNA processing at sites A_0, A_1, and A_2: U3 and U14, which are associated with Nop58p (27, 41a, 45, 80), and snR30 and snR10, which are associated with Cbf5p (40, 53, 70). Among these, the rRNA processing phenotype observed in the *cbp* deletions strains is most similar to this observed upon deletion of the *SNR10* gene (70). Depletion of Nop58p or Cbf5p leads to loss of the snoRNAs with which they are associated; moreover, several sno-

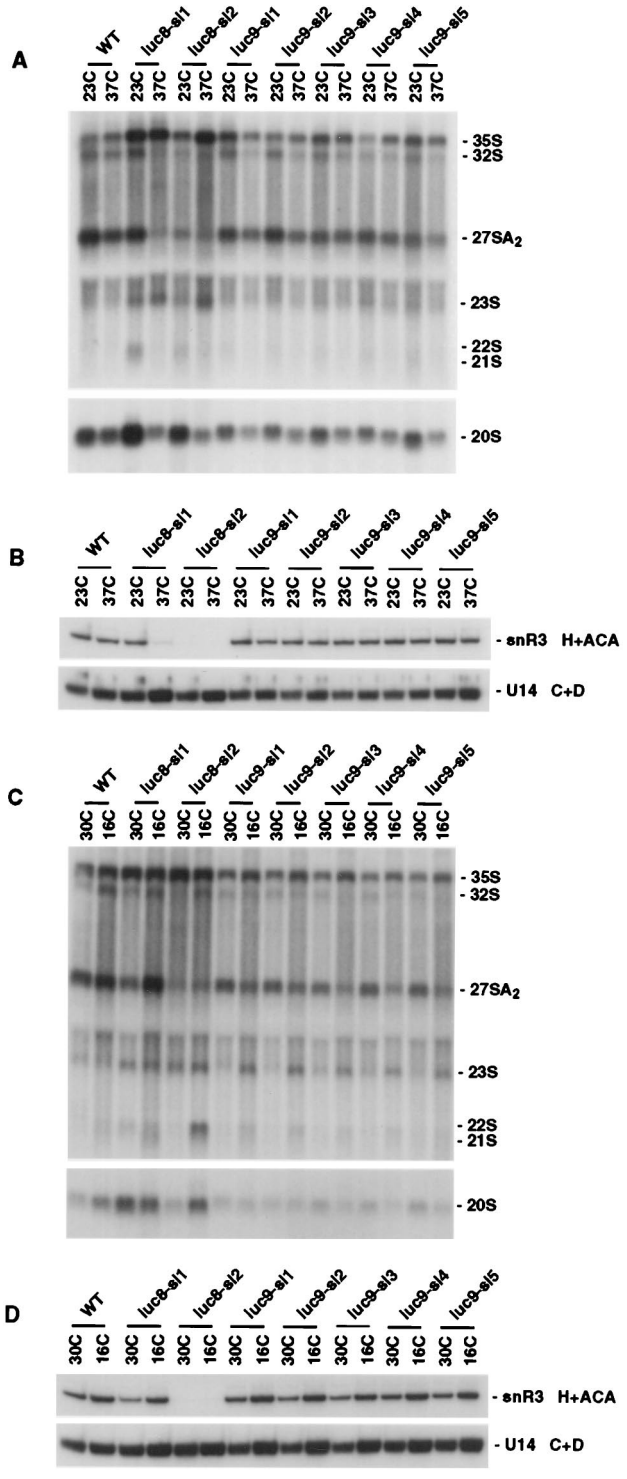


FIG. 5. Northern analysis of pre-rRNA (A and C) and snoRNA (B and D) levels in LUC8 and LUC9 strains. RNA was extracted following growth at 23°C and 18 h after transfer to 37°C (A and B) or following growth at 30°C and 12 h after transfer to 16°C (C and D). The oligonucleotide probes used in panels A and C were 003 (top) and 002 (bottom). WT, wild type.

RNAs, including U18 and U24, are encoded within pre-mRNA introns, and their synthesis could be inhibited by splicing defects. We therefore examined the steady-state levels of snoRNAs in the CBC deletion strains. No change in the steady-state levels of any of these snoRNAs was observed (Fig. 7 and data not shown).

Many snoRNAs, including U3, snR10, and snR30 snoRNAs, carry hypermethylated 5′ cap structures (26, 53, 79). The cap structures on the U3 and U8 snoRNAs have been reported to be required for nucleolar localization (28; but see reference 42) and therefore, presumably, for function. The efficiency of cap hypermethylation in the *cbp* strains was assessed by immunoprecipitation using a $m^{2,2,7}G$ cap-specific serum (R1131) and an monoclonal antibody that reacts with both $m^{2,2,7}G$ and m^7G cap structures (H20; kindly provided by R. Lührmann). No difference in immunoprecipitation was observed between RNAs extracted from the wild-type and *cbp20*-Δ strains, suggesting that the *cbp* strains were not deficient in snoRNA cap hypermethylation (data not shown).

Defects in ribosome assembly caused by inefficient splicing of the pre-mRNAs encoding ribosomal proteins can inhibit pre-rRNA processing in yeast (references 8 and 58 and references therein). Since processing defects were detected mainly in the small ribosomal subunit rRNA, we first investigated the splicing of small subunit ribosomal protein (*RPS*) pre-mRNAs in the CBC deletion strains. As a control, we utilized the temperature sensitive *prp2-1* strain, which exhibits a strong splicing block at the nonpermissive temperature (37°C) and consequent accumulation of pre-mRNAs (reference 58 and Fig. 8). Since CBC plays roles in the U1 snRNP-5′ splice site interaction and commitment complex assembly (10, 43, 44), we initially analyzed pre-mRNAs with nonconsensus 5′ splice sites (73). *RPS9A* and *RSP9B* contain GUACGU instead of GUA UGU; while *RPS11A* and *RPS11B* contain GUAUGA instead of GUAUGU (Fig. 8B).

Analysis of the steady-state levels of these mRNAs shows that splicing of the pre-mRNAs is inhibited. A similar degree of splicing inhibition was observed in the single and double *cbp* deletion strains (Fig. 8A, I to VI), while the different pre-mRNAs showed various degrees of inhibition. The level of *RPS11A* mRNA was not significantly altered, and there was no detectable accumulation of nonspliced pre-mRNA. By contrast, the mature *RPS9A*, *RSP9B*, and *RPS11B* mRNAs were depleted in the deletion strains and pre-mRNAs accumulated (Fig. 8A, I to VI; Table 2). The accumulation of unspliced precursors indicated that splicing of these pre-mRNAs is indeed defective. The defects in the splicing of *RPS9A* and *RPS11B* were particularly strong. These differences may be explained by examination of the pre-mRNA sequences. In addition to nonconsensus 5′ splice sites, *RPS9A* and *RPS11B* also lack optimal polypyrimidine tract and branchpoint region sequences (CACUAAC and GACUAAU, respectively, instead of UACUAAC). Since reporter introns with either 5′ splice site or branchpoint mutations are very poorly spliced in strains lacking yCBC (references 10 and 15a), this may contribute to their inefficient splicing in the absence of CBC. The splicing of actin pre-mRNA (Fig. 8A, XVIII) was not altered in the yCBC deletion strains. *RPS3* encodes a small subunit ribosomal protein but does not contain an intron (Fig. 8A VII), while the introns in *RPS10A* and *RPS10B* (data shown only for RPS10A [Fig. 8A, VIII]) have a consensus 5′ splice site. For each of these genes there was a clear decrease in mRNA, although this was not accompanied by an increase in the *RPS10A* or *RPS10B* pre-mRNAs (data not shown).

Since the levels of many pre-mRNAs and mRNAs encoding small subunit ribosomal proteins were affected in *cbc* strains, we examined whether this would also be the case for large ribosomal subunit (*RPL*) pre-mRNAs. The 5′ splice sites of these pre-mRNAs are GUACGU for *RPL16A* and *RPL16B*, GUAUGA for *RPL22A*, GUACGU for *RPL22B*, and GUC

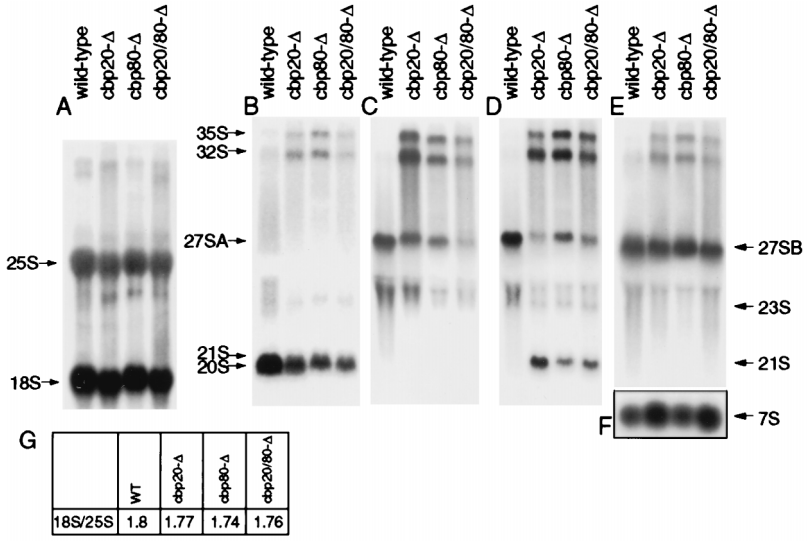


FIG. 6. yCBC is required for normal pre-rRNA processing. For Northern blot analysis of mature and precursor rRNAs, RNA was extracted from wild-type (WT) and *cbp* strains as indicated. (A) Hybridization with a probe complementary to the mature 18S and 25S RNAs; (B) hybridization with a probe complementary to the 5′ region of ITS1 (oligonucleotide 002); (C) hybridization with a probe complementary to the 3′ region of ITS1, downstream of site A_3 (oligonucleotide 001); (D) hybridization with a probe complementary to the central region of ITS1, between sites A_2 and A_3 (oligonucleotide 003); (E and F) hybridization with a probe complementary to the 5′ region of ITS2 (oligonucleotide 013). (G) Ratios of steady-state levels of mature 18S and 25S rRNAs. The positions of mature and precursor rRNA species are indicated; 21S and 20S pre-rRNAs are not well resolved; the identity of the 32S intermediate was verified by hybridizing a riboprobe complementary to the region between sites A_0 and A_1. Positions of the oligonucleotide probes are depicted in Fig. 4.

AGU for *RPL30*, compared to the consensus GUAUGU (Fig. 8B). The steady-state levels of these mRNAs were also decreased in all *cbc* strains, particularly for *RPL16A* and *RPL16B* (Fig. 8A, IX-XIV; *RPL16B* and *RPL22B* were very similar to *RPL16A* and *RPL22A* [data not shown]). The decrease in the level of mRNA was clearly accompanied by accumulation of pre-mRNA only in the case of *RPL30* (Table 2). Note that the 5′ splice site of *RPL30* pre-mRNA has two nonconsensus residues. *RPL25* and *RPL28* (*CYH2*) pre-mRNAs have consensus 5′ splice sites, whereas *RPL10* pre-mRNA has no intron. There was no significant reduction in the levels of these three mRNAs in the *cbc* strain. We conclude that the splicing of the pre-mRNAs with suboptimal splice sites is strongly inhibited in strains lacking CBC. The degree of inhibition varies between different pre-mRNAs with weak 5′ splice sites (Fig. 8A; Table 2), reflecting the differing relative levels of importance of CBC function in the splicing of those pre-mRNAs. Note that the reduction in some ribosomal protein mRNAs without concomitant increase in pre-mRNA, and the reduction in mRNAs from genes without introns, could well be a consequence of the impaired growth of the strains and consequent reduction in ribosome synthesis.

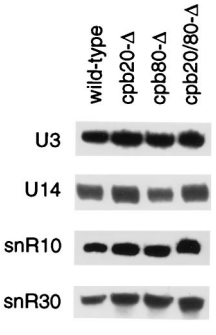
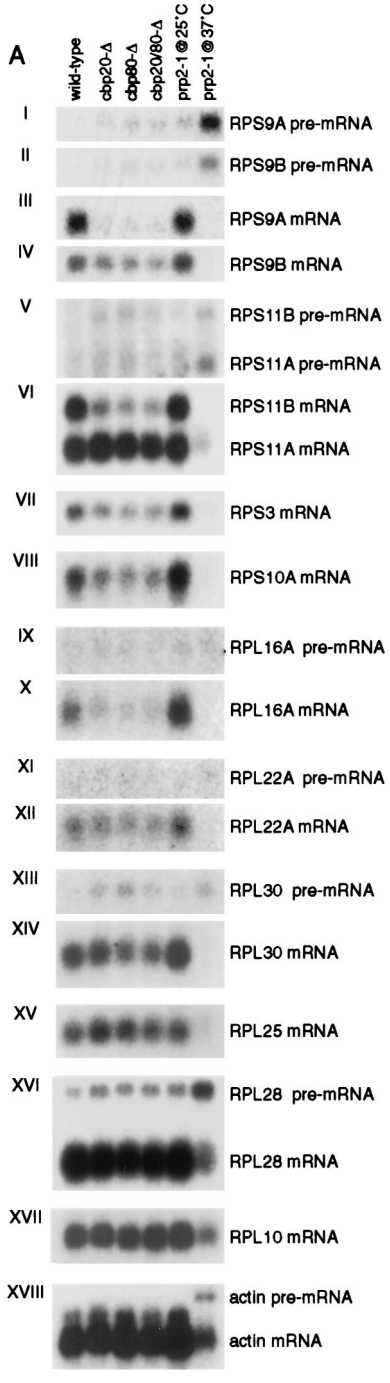


FIG. 7. yCBC does not affect accumulation of various snoRNAs. For Northern blot analysis of low-molecular-weight RNAs, RNA was extracted from wild-type and *cbp* strains as indicated. The probes used for hybridization are described in Materials and Methods.

Other SL complementing genes. LUC11 (Table 1) is complemented by *GCR1*, a transcriptional activator required for expression of multiple genes involved in glucose metabolism (3, 25). Since the gene encoding yCBP80, *GCR3*, was first identified in a search for additional mutants that affected growth on glucose (75), the finding of *gcr1* mutants in our screen was not a surprise. As would be expected, the alleles of *GCR1* recovered from the synthetic lethal screen did not cause lethality when the strains were plated on nonfermentable carbon sources (data not shown). Three additional complementation groups among the seven for which no complementing plasmid was recovered also failed to produce lethality when grown on nonfermentable carbon sources, suggesting that additional genes involved in glucose metabolism are likely to be involved in producing the SL phenotype. A possible explanation for both the earlier and present findings with *GCR1* is the report that the *GCR1* gene includes an intron with a nonconsensus 5′ splice site (GUAUGA instead of GUAUGU [73]).

There is no obvious reason why *SRV2* (LUC12) or any of the genes on the LUC14 complementing plasmid (Table 1) should, when mutated, generate a lethal phenotype in the absence of CBC. Similarly, although it is possible to speculate on possible functional connections between pol III transcripts (e.g., U6 snRNA) and CBC, the identity of any of the genes that complement LUC13 is not readily explicable. Given the role of CBC in U snRNA transport in vertebrates (31) and the existence of an abundant complex in yeast between CBC and yeast importin α (Srp1p), a mediator of nuclear protein import (20), it was of interest that the temperature-sensitive allele of LUC10/*SSD1* recovered in this screen accumulates poly(A)-containing RNA in the nucleus at nonpermissive temperature (data not shown). LUC10/*SSD1* was the only strain isolated in the screen showing this phenotype. However, an *ssd1*-Δ allele with a precise deletion of the entire ORF did not exhibit



nuclear poly(A) accumulation (21a). Sequence analysis suggests that *SSD1* likely encodes an exonuclease of the RNase II family (76), consistent with a role in RNA metabolism, but no change in mRNA stability was detected in either the *cbp* deletion strains or *luc10-1* strain (15a).

DISCUSSION

An extensive genetic analysis has been carried out with yCBC. Although there is strong evidence that vertebrate CBC is multifunctional (see the introduction), all of the genetic interactions with yCBC that are readily explicable are consistent with the function of yCBC at the commitment complex assembly stage of yeast pre-mRNA splicing (10, 43). Two strong candidates for direct physical interaction with yCBC in the commitment complex were identified: Mud10p, which does not yet have an identified vertebrate homologue; and Mud2p, the yeast U2AF65 homologue (1). Examination of interaction between human CBC and U2AF65, using techniques similar to those used here, indicate that these proteins also interact in vitro (15a). Surprisingly, however, the SR repeat-containing domain of U2AF65 (83) which is not conserved in yeast Mud2p (1) is the region required for this interaction. Further investigation of this interaction is in progress.

The approach used here, that of screening for genetic interactions that produce synthetic lethality, has previously been used successfully to identify several components of the yeast commitment complex (1, 2, 10, 46), and our analysis provides a strong confirmation of the usefulness of the approach in this case. Like the nuclear pore complex (12) or the Srb complex that forms part of the basal pol II transcription machinery (36), the commitment complex consists of a large number of components held together by multiple individual interactions, many of which may be relatively weak. Such complexes appear to represent particularly sensitive, and therefore productive, targets for this form of genetic analysis. This is presumably because a mutation that affects an individual interaction is often insufficient to destabilize the whole complex, whereas disruption of multiple combinations of two interactions will cause destabilization.

An interesting aspect of our data concerns the role of CBC in commitment complex formation and function. Based on previous data on vertebrate splicing, CBC has been viewed functionally as a cofactor that increases the interaction between U1 snRNP and the cap-proximal 5′ splice site (44). The yeast equivalent of this function might be fulfilled by the CBC-Mud10p interaction. Other results presented here suggest the yCBC function may be more complicated. First, both genetic and physical interaction between CBC and Mud2p were observed. Mud2p is not required for the interaction of U1 snRNP with pre-mRNA during formation of the initial commitment complex, CC1 (1); rather, it is needed for CC2 formation. Since Mud2p binds to the pyrimidine tract of the intron, i.e., 3′ of the BBP-branchpoint region complex (2, 7), this might suggest that yCBC's role extends beyond U1 snRNP function.

FIG. 8. yCBC affects steady-state levels of mRNAs of ribosomal proteins. RNA was extracted from either wild-type or *cbp* strains as indicated. Additionally, control RNA was extracted from a temperature-sensitive-lethal splicing-deficient *prp2-1* strain grown either at the permissive temperature (25°C) or after shift to the nonpermissive temperature (37°C) for 60 min. (A) Analysis of *RPS* and *RPL* mRNAs and pre-mRNAs. The positions of mature and precursor mRNAs are indicated. Probes used for hybridization are described in Materials and Methods. (B) Sequences at the 5′ splice sites of pre-mRNAs analyzed in this study. Nonconsensus residues are underlined. All pre-mRNAs tested contain one intron, and in all cases it is located close to the 5′ end of the pre-mRNA.

TABLE 2. PhosphorImager (Molecular Dynamics) analysis of the accumulation of *RPS9A*, *RPS11B*, *RPL30*, and *RPL28* pre-mRNAs and mRNAs[a]

Strain	*RPS9A*			*RPS11B*			*RPL30*			*RPL28*		
	Pre-mRNA	mRNA	Pre-mRNA/mRNA	Pre-mRNA	mRNA	Pre-mRNA/RNA	Pre-mRNA	mRNA	Pre-mRNA/mRNA	Pre-mRNA	mRNA	Pre-mRNA/mRNA
Wild type	1	65.05	0.015	1	10.27	0.1	1	14.3	0.07	1	13.7	0.073
cbc20-Δ	5.16	6.3	0.82	4.08	2.1	1.94	1.58	10.4	0.15	1.34	12.6	0.11
cbc80-Δ	9.76	9	1.08	4.11	2.25	1.83	1.89	7.33	0.26	1.35	10.45	0.13
cbc20/80-Δ	7.65	9.69	0.77	3.43	2.18	1.57	1.66	7.8	0.2	1.31	11.27	0.12
prp2-1												
25°C	10.22	66.08	0.15	3.79	10.33	0.37	1.37	18.4	0.074	1.38	14.2	0.097
37°C	50.7	7.05	7.2	2.1	0.14	15	1.98	1.29	1.53	3.9	2.26	1.73

[a] Values relative to those of wild-type pre-mRNA are shown.

Further evidence for this hypothesis comes from comparing the efficiencies of splicing between *RSP9A* and *RSP9B* pre-mRNAs and between *RSP11A* and *RSP11B* pre-mRNAs. These two pairs of pre-mRNAs exhibit markedly different splicing efficiencies in the absence of CBC. These differences do not correlate with differences at the 5′ splice site, where U1 snRNP interaction occurs, but with differences in the pyrimidine tract regions of the introns. These results might suggest that yCBC stabilizes both U1 snRNP-5′ splice site and Mud2p-3′ splice site interactions.

CBC and pre-rRNA processing. An unexpected result from these analyses was the identification of five SL strains that could be complemented by *NOP58* and two that were complemented by *CBF5*. These genes encode essential nucleolar proteins that are core components of the box C+D and box H+ACA families of snoRNPs, respectively (reviewed in reference 41). Nop58p and Cbf5p are both required for the early pre-rRNA processing steps at sites A_0, A_1, and A_2 on the pathway of 18S rRNA synthesis. The SL strains were each found to have defects in pre-rRNA processing at these steps, in the presence of functional CBC. Similarly, strains lacking CBC were found to be defective in the cleavage of sites A_0, A_1, and A_2, with the greatest effect on site A_2. Synergistic inhibition of rRNA synthesis is therefore likely to underlie the observed SL interactions. A large number of genes encode pre-rRNA processing factors, and it is unclear why only two complementation groups were isolated in multiple strains. One possibility is that snoRNAs with which Nop58p and Cbf5p associate also play roles in the modification of spliceosomal snRNAs and therefore participate, indirectly, in pre-mRNA splicing. Box C+D snoRNAs guide 2′-O-methylation of several positions in the U6 snRNA in vertebrates (74); however, equivalent guide RNAs have not been identified in yeast.

Strains lacking CBC were found to be defective in the splicing of pre-mRNAs that encode ribosomal proteins, particularly those in which the sequences at both the 5′ and 3′ ends of the intron were nonconsensus. The pre-rRNA processing defect in the *cbc* mutants may therefore be a consequence of reduced, or imbalanced, ribosomal protein synthesis. In the *cbc* strain, the steady-state level of the mature rRNAs was not clearly altered, suggesting that reduced rRNA synthesis is not the direct cause of the growth defect. This phenotype resembles that seen in strains lacking the snoRNA, snR10 (70). Like Cbp20p and Cbp80p, snR10 is not essential, but its absence impairs cell growth. Pre-rRNA processing is inhibited at sites A_0, A_1, and A_2, with the greatest effect on A_2. Accumulation of mature rRNA is, however, not prevented, and the impaired growth is probably due to a defect in ribosome assembly (77). We speculate that an alteration in the stoichiometry of the ribosomal proteins interferes with normal ribosome assembly, leading to the synthesis of partially defective ribosomal subunits.

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